COMMUNICATIONS IN PLANT SCIENCES

RESEARCH ARTICLE

Response of rice genotypes subjected to salt stress

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Soils with high salt concentrations (NaCl) may affect plant development and nutrient uptake in rice. The objective of this experiment was to evaluate the response of rice cultivars released by Embrapa Clima Temperado to salt stress at the germination and seedling stages. Four NaCl concentrations (0, 40, 80 and 120 mM) and a random factorial design 4×6 (dose × genotype), the cultivars belonging to the Embrapa germplasm collection were evaluated, BRS Bojuru (the only tolerant variety), BRS Pampa, BRS 7 Taim, BRS Querência, BRS Atalanta and BRS Sinuelo CL, with three replicates. After 14 days, the levels of Na, K, Ca and Mg absorbed by the plant shoots and roots were determined. NaCl was not significantly present in the germination stage. In the first germination count, BRS Bojuru and BRS 7 Taim decreased the germination with the increase in salt. The length of the first leaf in the 40 mM dose at 14 days of development (vegetative stage) is indicated as morphological marker for discriminating sensitive and tolerant genotypes to salinity in hydroponic system. The cultivar BRS Pampa showed higher ability of leaf Ca and Mg translocation under salt stress conditions.

Highlighted Conclusions

1. The genotypes have contrasting responses in relation to the increase of NaCl doses, due to the interaction G x D for all evaluated variables.
2. The length of the first leaf in the 40 mM dose at 14 days of development (vegetative stage) is indicated as morphological marker for discriminating sensitive and tolerant genotypes to salinity in hydroponic system.

The Asian rice (*Oryza sativa* L.) is widely cultivated in the world, reaching a large economic and social importance. Brazil produces over 11 million tons of rice annually, the majority amount under irrigated (flooding) system. The State of Rio Grande do Sul is the major Brazilian producer, contributing to ca. 70% of the production of this cereal (Conab 2016).

Soil and irrigation water salinity are major sources of salt stress, a major abiotic factor reducing irrigated rice yields in several parts of the world (Sobhanian et al. 2011). This scenario can be observed in Rio Grande do Sul state, mainly in coastal regions and farming systems where inadequate drainage combined with poor quality water for irrigation predominate.

Harsh conditions causing biotic and abiotic stresses may provide morphological, physiological, biochemical and molecular changes in plants, negatively affecting their growth, reproduction and agricultural production. Plants react to stress conditions according to their genetic potential, developing efficient mechanisms to quickly adapt to stressful conditions (Mahajan and Tuteja 2006, Yang et al. 2012). Plants have showed complex changes in the limbs, cells and metabolic processes, increasing levels of salinity tolerance (Zhang et al. 2011).

Soil salinity may be due to its formation process. However, in order to have suitable water for irrigation, one should consider the water salinity degree (expressed by the Na^+ content) and soil salinity (saturation expressed by Na^+ exchange complex) (Sosbai 2010). The excess salt in the irrigation water may cause further damage to the crop, providing Na^+ accumulation in the soil. A high concentration of Na^+ in the soil may impair the absorption of other cations, such as Ca^{2+}, Mg^{2+} and K^+, by competition for the absorption sites in the rice roots.
Rice is considered to be particularly sensitive to salinity in the vegetative and reproductive stages. However, it is one of the few crops that can flower in soils affected by salts due to its ability to grow in flooded areas. It is even recommended as a crop for the desalination of excess salts in the soil (Ismail et al. 2007, Singh et al. 2010). Many rice cultivars, currently used in Rio Grande do Sul, are sensitive to salinity, at both seedling and reproductive stages.

Seed germination is a fundamental step in agricultural production. High levels of salinity significantly influence the germination process damaging the crop establishment. Its disorder is due to a combination of effects such as ionic imbalance, hyperosmotic stress and oxidative damage, causing reduction in the amount of water absorbed by the seed germination process affecting their performance (Ruan et al. 2011).

Response to salinity is genotype dependent. Even though a range of sensitivity is seen among plants, high salinity levels cause water deficit and ion toxicity in many species (Sobhanian et al. 2011).

Besides the species and the genotype within species, the development stage and environmental factors are critical for the discrimination between tolerant and sensitive genotypes. Tolerance to salinity seems to be more frequent in japonica than in indica genotypes (Machado and Terres 1997).

The aim of this study was to evaluate the performance of released Embrapa rice cultivars regarding to their tolerance to salt stress in germination and seedling stages.

MATERIAL AND METHODS

Plant Material. Six rice cultivars belonging to the Embrapa germplasm collection were evaluated: BRS Bojuru (the only tolerant variety), BRS Pampa, BRS 7 Taim, BRS Querência, BRS Atalanta and BRS Sinuelo CL. These genotypes were chosen because of their agronomic and scientific interest. Genotypes were tested for salinity stress in two stages: i) germination stage on a germinating chamber (BOD) and ii) seedling stage on hydroponic culture under controlled environmental conditions. The experiments were carried out at the Plant Genomics and Breeding Center (CGF), School of Agronomy "Eliseu Maciel "(FAEM), Federal University of Pelotas (UFPel), Capão do Leão - RS, Brazil.
Growth conditions. For the germination analysis and the first count of germination, 50 seeds of each genotype were germinated in germination boxes, using germitest paper with an amount of water equivalent to 2.5 times the substrate weight on germinating chamber (BOD) at 25 °C with a 16 hours photoperiod and 100% relative humidity, according to the criteria established by the Rules for Seed Analysis (Brasil 2009). For the hydroponic evaluation, seedling with uniform root length (around 5 mm) were prepared on polyethylene nets adapted to plastic containers, allowing the support and seedling root growth into the medium. This system maintains the root in permanent contact with the nutrient solution (Yoshida et al. 1976). The containers were placed in a hydroponic system in a water bath (25±1 °C) with controlled lighting (16 hours photoperiod). The nutrient solution was not changed, only distilled water was added to compensate evaporation.
Design experimental. The experiment was conducted in randomized block design with four replications in a 4×6 factorial scheme with genotype × dose. The first count of germination and germination were performed at 7 and 14 days, respectively, and expressed as percentage of normal seedlings. The treatments consisted of four NaCl concentrations (0, 40, 80 and 120 mM) added to the nutrient solution. After preparation of the nutrient solution, a pH adjustment to 4.5 was made. The concentrations were previously selected through studies made by Khan et al. (1997), which reported that salinity tolerance in plants can occur on germination and seedling stages differently.
Traits available. The plants were analyzed at 7 and 14 days, through the evaluation of the following variables: number of roots (NR), root length (RL) in cm, length of shoot (SL) in cm; first leaf insertion (FLI), in cm; length of the first leaf (FLL) in cm, and a length of the second leaf (SLL) in cm.

After 14 days, shoots and roots of plants were collected for the determination of sodium (Na), potassium (K), calcium (Ca) and magnesium (Mg) absorbed.

For these determinations, the plants were oven-dried at 65 °C until reaching constant weight, afterward they were weighed and milled. The macronutrient extraction (K, Ca and Mg) was obtained by wet sulfuric acid digestion of plant tissue (Tedesco et al. 1995). Due to the sodium presence in the digesting solution described above, the sodium extraction was made with another method described by Castilhos (1982). In this digestion successive applications of 0.2 mL of hydrogen peroxide (H_2O_2) were added. The block temperature was maintained at around 140 °C. The contents of Na, K, Ca and Mg were quantified in extracts by flame photometry. The amounts of Na, K, Ca and Mg accumulated by plants were obtained based on these element concentrations and the amount of dry matter. Nutrient absorption levels were calculated as relative performance (increase or decrease) in percentage, considering the absolute value of the control (0 mM of NaCl) as 100%.

Statistical analysis. The results were subjected to variance analysis ($p \leq 0.05$) and for those that tested significantly polynomial regression models were tested using WinStat statistical program (Machado and Conceição 2007). The models were chosen based on the statistically significant (F test) and determination coefficient (R^2). For plotting the figures, the Microsoft® Office Excel 2007 program was used.

RESULTS AND DISCUSSION

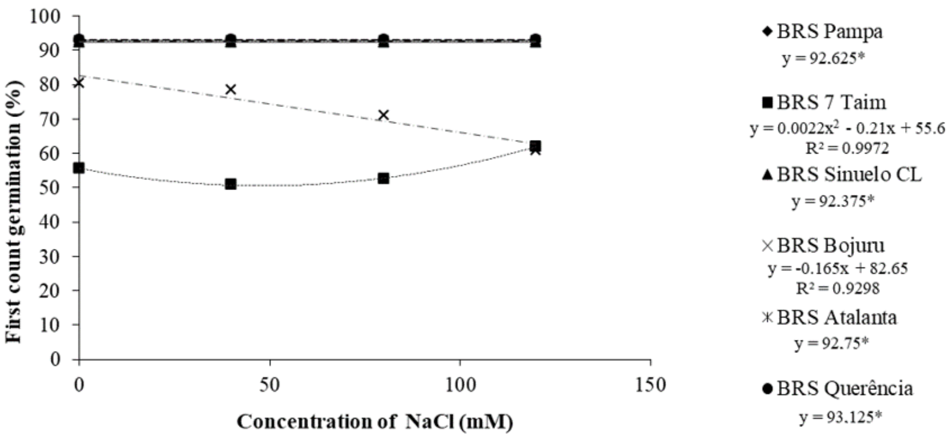
Through the analysis of variance, it can be observed that the salinity effects on seed germination were not significant for dose and the interaction between genotype and dose. In some cases, rice has salt tolerance at the germination stage (Khan et al. 1997). Significant effects for genotype and genotype x dose at the first count germination were found (Table 1).

Table 1. Summary of analysis of variance, mean and coefficient of variation for the first count germination variables (FCG) and germination (G), of six rice genotypes, valued at four NaCl concentrations (0, 40 mM, 80 mM and 120 mM).

Source of variation	DF	Means Square	
		FCG	G
Genotype	5	4009.94*	3771.61*
Dose	3	48.71	68.54
Genotype x Dose	15	79.54*	105.32
Residue	72	23749.96	105.22
Overall average	−	83.15	84.80
CV (%)	−	6.89	12.09

*Significant values at p ≤ 0.05 by F test.
DF= degrees of freedom CV= coefficient of variation

The cultivars BRS Pampa, BRS Querência, BRS Atalanta and BRS Sinuelo CL showed the same response in all treatments, not expressing phytotoxicity symptoms caused by excess salt. While BRS Bojuru was fit to a linear regression model showing a decrease in the percentage of first count germination, with increasing NaCl concentrations, BRS 7 Taim was fit to a quadratic regression, with a decrease in 40 mM dose, showing subsequent increase in the percentage of first count germination in the other tested doses (Figure 1).



* No significant equation at p ≤ 0.05

Figure 1. Regression equations parameters and their graphical representations of the first count germination (FCG) and germination (G) of six rice genotypes valued at four NaCl concentrations (0, 40 mM, 80 mM and 120 mM).

Seven days after treatment implementation, variance analysis revealed significant interaction genotype×dose effects for all variables (Table 2), indicating that the genotypes showed differential responses according to the applied NaCl doses. Figures 2 and 3 illustrate the variables responses to salinity action on the different genotypes. The genotypes showed differential responses to dose effects for all variables, confirming the results obtained in the analysis of variance that showed a significant effect for genotype × dose.

Table 2. Summary of variance analysis, mean and coefficient of variation for characters shoot length (SL), first leaf insertion (FLI), the first leaf length (FLL), second leaf length (SLL), root length (RL) and number of roots (NR), six genotypes of rice, valued at four concentrations of NaCl (0, 40 mM, 80 mM and 120 mM) at seven and 14 days of cultivation in hydroponic system.

Source of variation	DF	Means Square 7 days					
		SL	FLI	FLL	SLL	RL	NR
Genotype	5	69.34*	4.29*	12.62*	41.00*	61.88*	33.53*
Dose	3	3891.64*	154.42*	16.50*	1716.43*	3472.50*	759.55*
Genotype x Dose	15	23.11*	2.03*	2.00*	24.00*	30.91*	13.36*
Residue	696	1.72	0.16	0.16	1.35	2.53	2.10
Overall average	−	6.14	1.64	1.39	3.38	5.43	4.83
CV (%)	−	21.34	24.50	29.08	34.40	29.30	29.97

		Means Square 14 days					
		SL	FLI	FLL	SLL	RL	NR
Genotype	5	749.97*	15.01*	4.75*	106.23*	445.74*	128.65*
Dose	3	8782.38*	259.94*	112.87*	1683.42*	5673.24*	2345.57*
Genotype x Dose	15	298.39*	7.98*	5.41*	52.94*	219.57*	63.47*
Residue	696	11.17	0.57	0.62	3.19	11.26	4.03
Overall average	−	5.70	1.05	0.77	2.58	4.78	2.98
CV (%)	−	58.67	71.69	101.52	69.14	70.11	67.43

* Significant values at $p \leq 0.05$ by F test
DF= degrees of freedom CV= coefficient of variation

The variables length of shoots (ls) and insertion of the first leaf (insfl), indicated a quadratic regression model fitting for most genotypes, except for BRS Bojuru, which showed linear regression (Figure 2). The genotypes that have adapted to the quadratic regression model showed a decrease with increasing salt concentration, showing a coefficient of reduction of 0.0643 and 0.0101, respectively for ls and insfl. The variable length of the first leaf (Figure 2), displayed a quadratic regression model response for BRS 7 Taim and BRS Bojuru. This suggests an increase in tolerance to salt up to 80 mM and a decrease thereafter. The other cultivars also have fitted to a quadratic regression model response, but showed sensitivity to salt.

The results for the morphological character second leaf length (Figure 3) indicate that most genotypes were fit to a quadratic regression model, with the exception of BRS 7 Taim (linear regression). The graphical analysis reveals the tolerance reaction for BRS Bojuru which differed from the other genotypes analyzed, showing a less steep decrease, with a 0.0145 reduction coefficient.

The characters root length and number of roots (Figure 3) showed a different response between the sensitive and the tolerant cultivars, allowing their differentiation. Again, BRS Bojuru showed a less steep decrease than the remaining genotypes for both traits.

Figure 4 and 5 illustrate the variables responsiveness to the action of the salt on the genotypes at 14 days after the experiment set up. It was found that the genotypes showed differential responses to dose effects for all variables, agreeing with the results obtained in the analysis of variance (Table 2).

As seen for other variables, for the variable shoot length (Figure 4), BRS Bojuru response fitted to a linear regression model, with lower reduction coefficient, demonstrating increased tolerance to the salt effects in this genotype.

For insertion of the first leaf and length of the second leaf (Figures 4 and 5), the genotypes showed similar responses. BRS Bojuru and BRS Pampa were fit to a linear regression model, but the tolerant genotype had lower regression coefficient. The remaining genotypes showed sensitivity to the salinity effect, fitting into a quadratic regression model. BRS Bojuru response, adjusted to quadratic regression model, showed a further reduction in the character evaluated. BRS 7 Taim and BRS Atalanta responses also were fit to the quadratic regression model, but had higher regression coefficients, characterizing sensitivity to NaCl. The other cultivars that were fit to linear regression also suffered the salt effects. These variables allow one to discriminate sensitive and tolerant genotypes.

The root length variable reveals differential response between sensitive and tolerant genotypes, establishing a group that fits to a quadratic regression model, with the exception of BRS Bojuru (tolerant). BRS Pampa, with quadratic regression, differs from other sensitive cultivars by presenting a lower reduction.

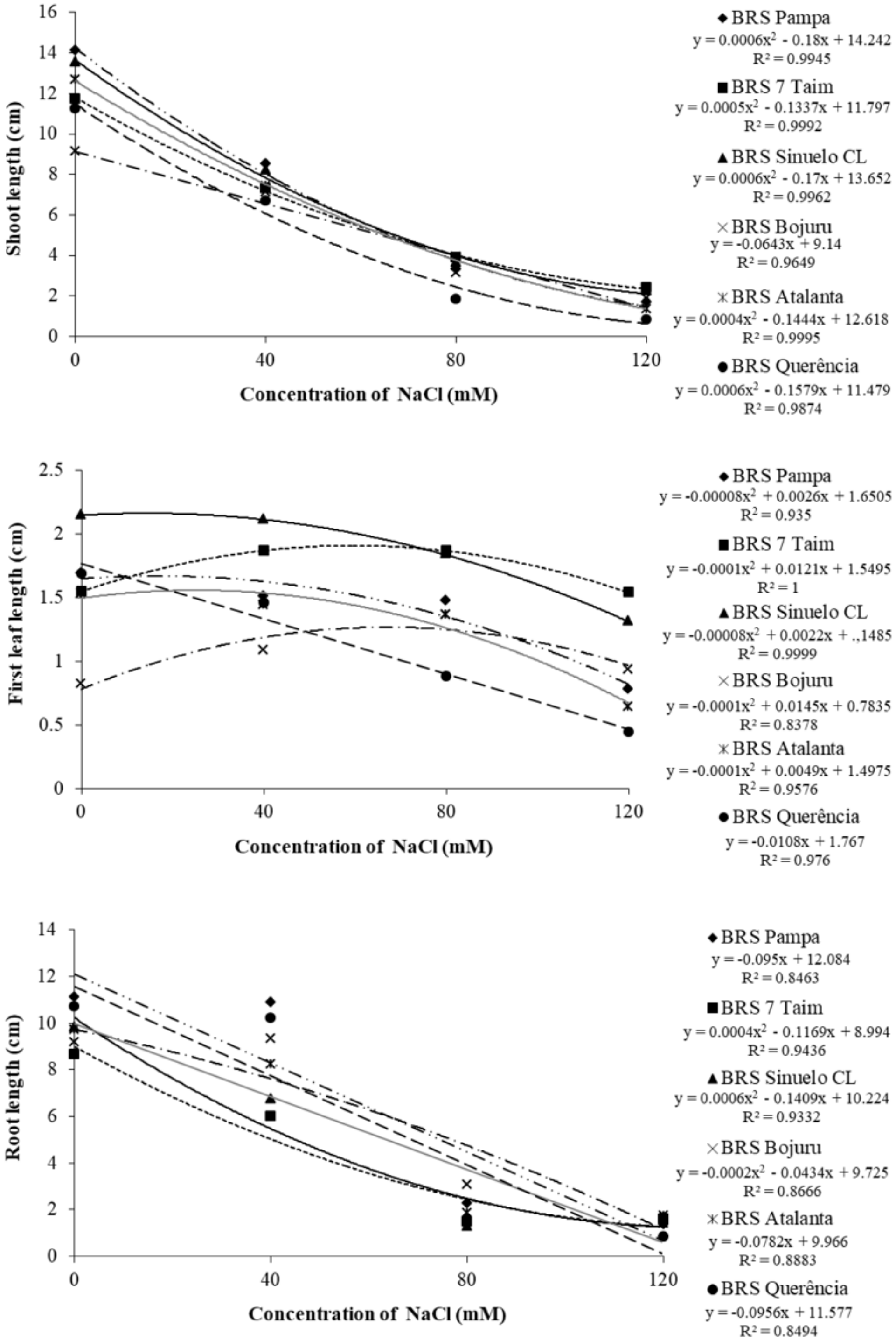


Figure 2. Graphical representations and regression equations parameters of the variables: shoot length (SL), first leaf length (FLL), root length (RL) of six rice genotypes valued at four NaCl concentrations (0, 40 mM, 80 mM and 120 mM) at seven days of cultivation in hydroponic system.

For the variable number of roots, a different response between sensitive and tolerant cultivars was observed (Figure 5). BRS Bojuru presented the best fit linear regression model, the remaining genotypes formed a group fitting a quadratic regression model, with a sharp reduction in the character evaluated.

The importance of phenotypic characterization of genotypes in the vegetative phase is emphasized by Lutts et al. (1995), which reported that rice exhibits high sensitivity at the seedling stage, which may affect the density and initial stand of the crop in saline soils. In the seedling stage, rice may notice signal salt stress through plasma membrane receptors on cells and the roots may initiate intracellular signaling which modulates gene expression to induce an adaptive response (Ruan et al. 2011).

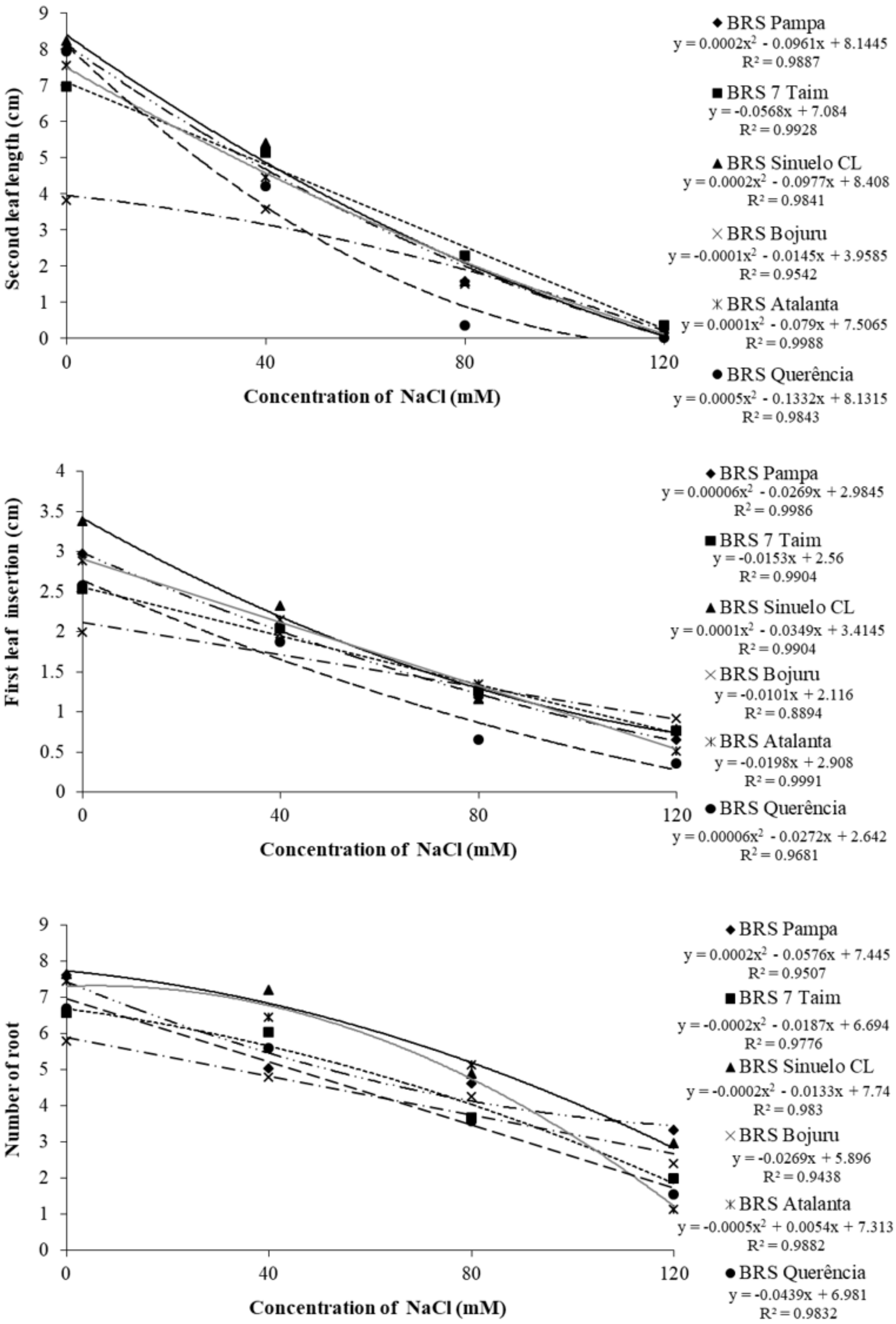


Figure 3. Graphical representations and regression equations parameters of the variables: second leaf length (SLL), first leaf insertion (FLI) and number of root (NR) of six rice genotypes valued at four NaCl concentrations (0, 40 mM, 80 mM and 120 mM) at seven days of cultivation in hydroponic system.

Roots play an important role in plant responses to abiotic stresses, being one of the first plant organs to detect environmental changes. When plants sense salinity, a kind of cell death program occurs, causing early leaf senescence, reducing the water demand, trying to soothe cellular stress (Zhou et al. 2013). In the suppression of this induced type of leaf senescence, plants would maintain higher water content and better photosynthetic activity during stress (Lutts et al. 1996, Rivero et al. 2007).

The variable length of the first leaf may be considered appropriate for studies because it discriminates more efficiently the performance of genotypes under different doses and developmental stages. The variable length of the first leaf at 14 days of seedling development can be indicated to be used as a marker for tolerance screening.

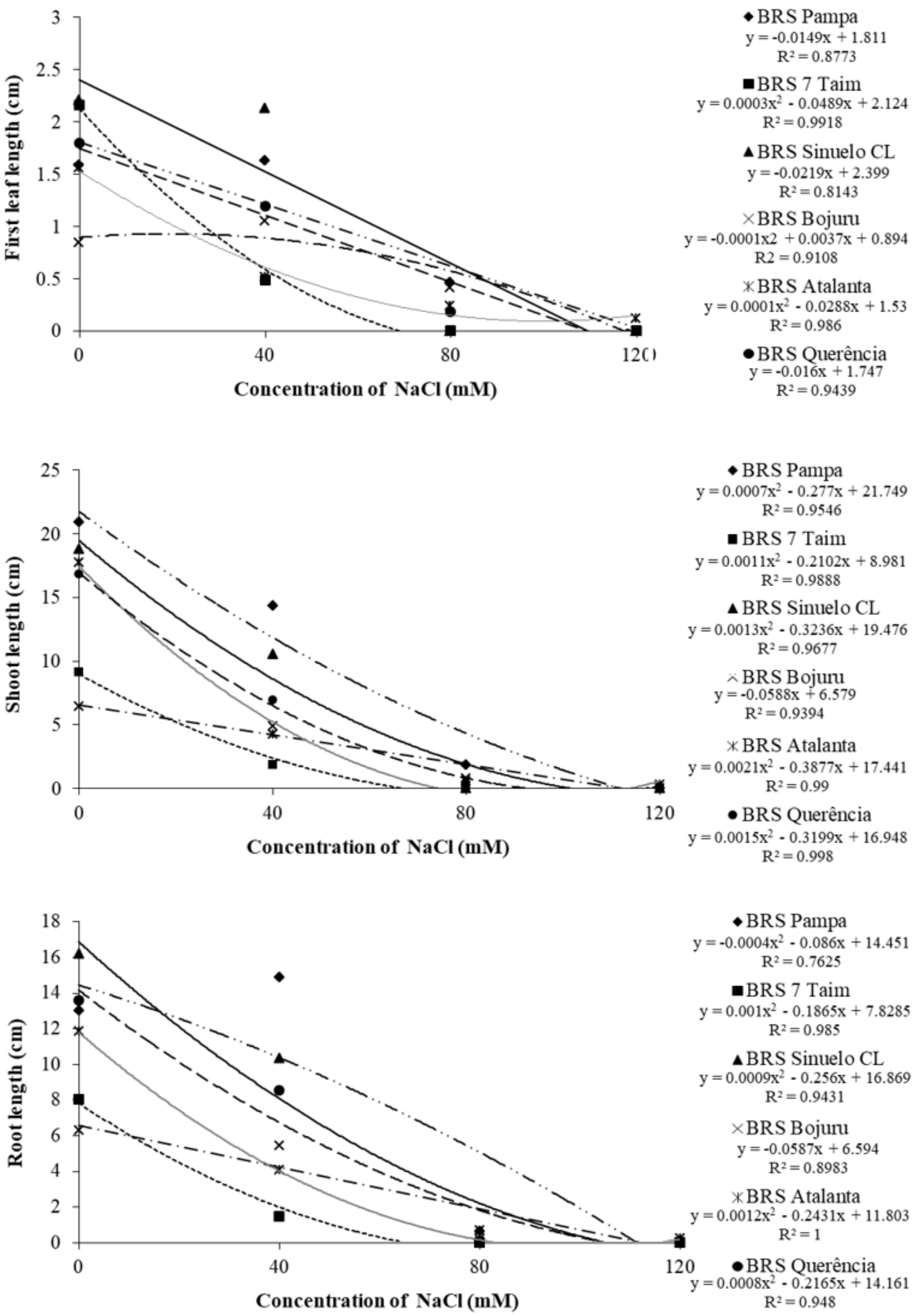


Figure 4. Graphical representations and regression equations parameters of the variables: shoot length (SL), first leaf length (FLL), root length (RL) of six rice genotypes valued at four NaCl concentrations (0, 40 mM, 80 mM and 120 mM) at 14 days of cultivation in hydroponic system.

The doses of 80 and 120 mM NaCl were lethal to the cultivars analyzed in hydroponic system at 14 days of cultivation. However, the 40 mM dose was sufficient to discriminate between sensitive and tolerant genotypes. It would be interesting to conduct experiments with doses smaller than we used to allow better genotype differentiation.

The analysis of nutrient content in the plants (Figure 6) describes the relative performance percentage (increase or decrease) in order to eliminate possible pH interference of the Yoshida's solution (Yoshida et al. 1976) in the absorption of macro and micronutrients analyzed and the NaCl influence on the absorption of elements studied.

The sodium content in the plant can be considered as a good indicator of toxicity level which cells are exposed. The lower stress tolerant genotypes, the smaller the number of genes that respond to salinity (Walia 2007).

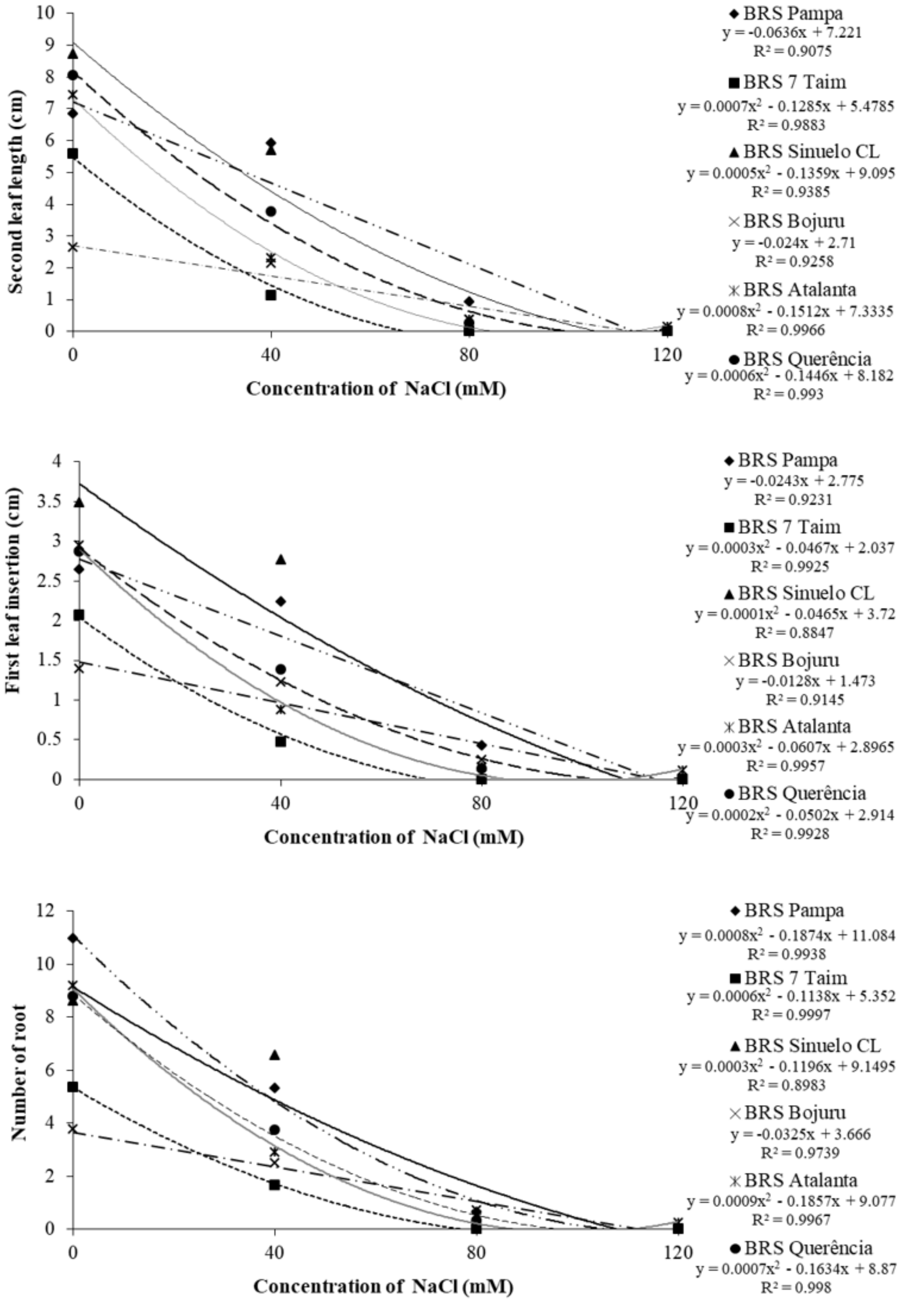


Figure 5. Graphical representations and regression equations parameters of the variables: second leaf length (SLL), first leaf insertion (FLI) and number of root (NR) of six rice genotypes valued at four NaCl concentrations (0, 40 mM, 80 mM and 120 mM) at 14 days of cultivation in hydroponic system.

Analyzing the relative performance percentage in Figure 6a, it can be inferred, regarding the Na content absorbed the six genotypes showed higher values than the absolute, as in the aerial part as root part of the plant. BRS Pampa showed higher Na levels in leaves and roots in all treatments.

The relative performance percentage to K content absorbed can be observed in Figure 6b. It presents, in general, a decrease with increasing NaCl concentrations for the genotypes. In addition, any cultivar was equal or higher than the control treatment (100%). The genotype BRS Bojuru (tolerant) showed similar K levels in the leaves and roots in all treatments, demonstrating better nutrients translocation to the leaves.

No genotype showed genetic superiority regarding relative performance for Ca content, measured in the leaves (Figure 6c). However, higher values than the absolute were observed for BRS Bojuru, BRS 7 Taim and BRS

Pampa at 80 mM NaCl. At 120 mM NaCl, only BRS Sinuelo CL did not show superiority over the control treatment. It can be observed that under salt stress conditions BRS Pampa showed the highest Ca levels at the doses tested, indicating better nutrient translocation to the leaves.

As shown in Figure 6d, the analysis of the relative performance for Mg content indicates an increase in the concentration in the roots of BRS Bojuru with the increase in NaCl concentration. No genotype exceeded the absolute value of the control treatment in both leaves and roots, suggesting a phytotoxic NaCl effect on rice plants. The BRS Pampa cultivar stands out with the Mg mobility absorption to the leaves, showing the highest levels at the doses tested.

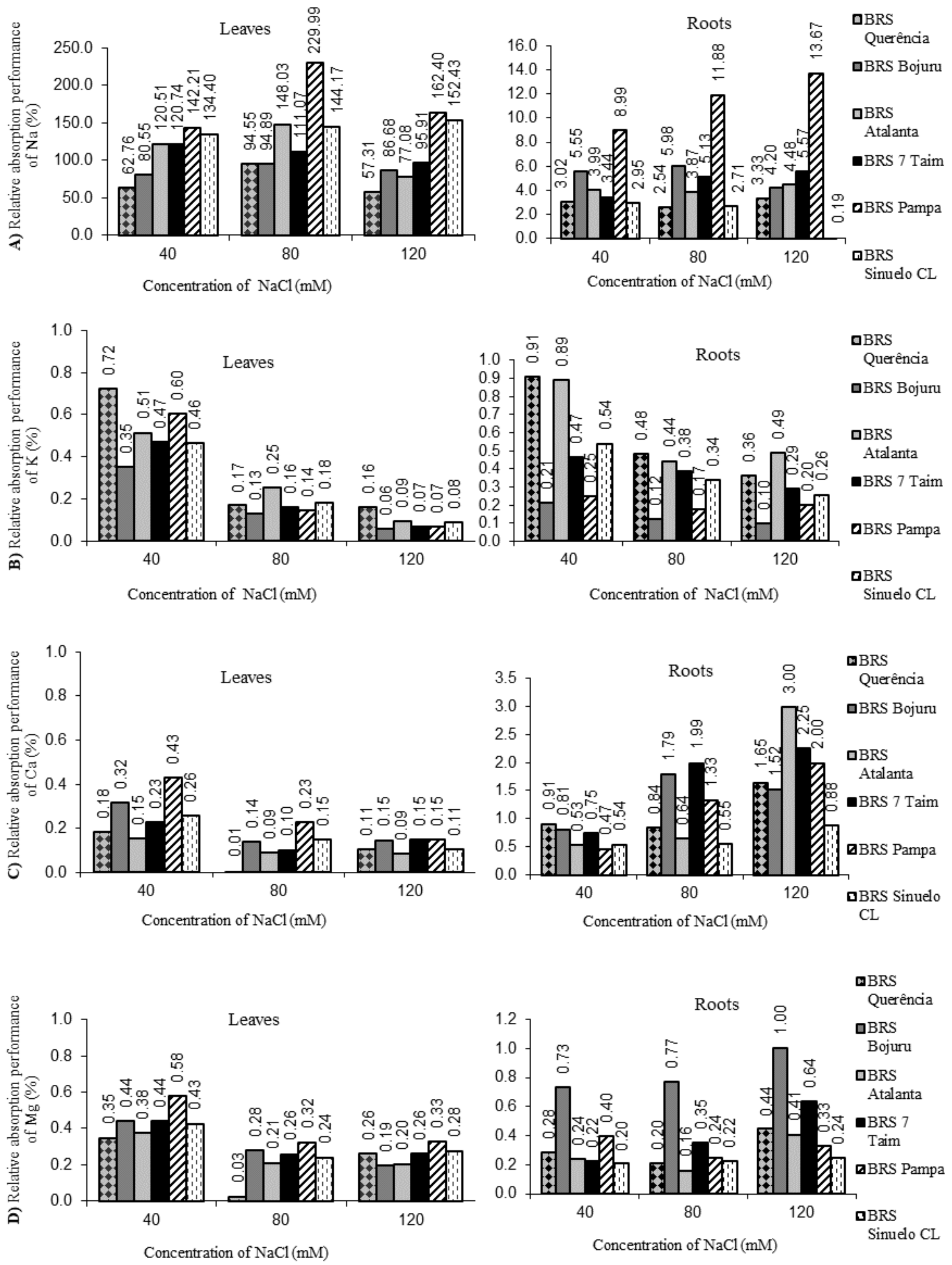


Figure 6. Relative performance of Sodium (Na), Potassium (K), Calcium (Ca) and Magnesium (Mg) absorption by leaves and roots of six rice genotypes valued at four NaCl concentrations (0, 40 mM, 80 mM and 120 mM) after 14 days of cultivation in hydroponic system, considering as reference the absolute value of the treatment corresponding to 100% control.

Among the genotypes, except for the Ca element in the roots, there was no superiority to the control treatment in the absorption of nutrients. For Na, all genotypes showed values above the control treatment in leaves and roots in all doses. BRS Pampa cultivar showed the highest levels of Ca and Mg in the tested doses, suggesting nutrient translocation to the leaves under salt stress conditions.

Well characterized phenotypic responses to salt stress in a certain growth stage can help to identify potential parents for the production of hybrid genotypes, through the selection of complementary and favorable alleles for salt tolerance, collaborating with breeding programs. An investigation of the physiological and molecular mechanisms underlying this salinity tolerance study will provide valuable information for effective genetic engineering strategies.

The main results this work are that toxicity effects caused by NaCl excess were not significant at the germination stage for all genotypes. The BRS Bojuru and BRS 7 Taim cultivars suffer reduction in vigor test first count with increasing NaCl concentrations. The character length of the first leaf is the most responsive variable and can be used as a morphological marker in experiments for selection of NaCl resistant rice varieties. The NaCl concentration that best discriminates sensitive and tolerant genotypes in a hydroponic system, in the two seasons studied, is 40 mM. The best time to discriminate the sensitive and tolerant genotypes to salt stress is 14 days under hydroponic system. The BRS Pampa cultivar stands out with its greater capacity for Ca and Mg translocation to the leaves under salt stress condition.

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